

STUDIES FOR STUDENTS.—III. ELEMENTARY STUDIES IN
INSECT HISTOLOGY.

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For the study of the anatomy, or histology, of insect tissues, the laboratory or working room must have a certain minimum of equipment and the student a certain elementary training in histologic method or technic. By the technic of insect histology is meant the particular methods of killing, fixing, hardening, clearing, infiltrating and imbedding, sectioning, staining, and mounting, so that the various body tissues may be available for examination and study under considerable microscopic magnification. With the methods of manipulation acquired by instruction and experience, the actual study of the histologic characteristics of the various particular tissues and organs of the insect body can be undertaken. The tissues should be studied first, for almost any organ comprises in its intimate make-up several distinct tissues or kinds of cellular aggregates. In this paper I purpose giving, first, a brief account of a generally applicable course of procedure in preparing insect tissues for histologic study, and then a series of brief directions and hints for the recognition and study of the various typical or normal insect tissues, and finally similar directions and suggestions for the study of the fine anatomy of the principal insect body-organs.

As in Studies I (PSYCHE, vol. 9, p. 207) on insect anatomy and Studies II (*Loc. cit.*, p. 246) on the development of the histoblasts of wings and legs, the giant crane-fly *Holorusia rubiginosa* was used for specific subject, the same insect species will be used as principal subject of this paper. But it is plain, that the similar study of any other insect may be based on the study here outlined of this particular one.

HISTOLOGIC TECHNIC. *Killing and fixing.*—The chitinized cuticula of the insect body is nearly impervious to fixing fluids, so that for quick killing and fixing of the tissues, heat is, in most cases, the best killing agent. Tissues that have been dissected out from the body of a live (chloroformed) specimen may be fixed without heat in any of the usual fluids. To kill and fix the whole body of insects, drop specimens alive into boiling water; leave them in this but a moment or two, *i. e.*, until the body is rigid, then transfer to 30% alcohol. While here puncture the body wall with a needle, scalpel, or fine scissors in several places, not cutting deeply nor making the wound in the dorso-ventral median longitudinal plane of the body. Leave in 30% alcohol three hours; then transfer to 50% alcohol for three hours, then to 75% alcohol for from six to twelve hours, then to 85% alcohol

in which the specimens may be preserved indefinitely. Or, transfer the specimens from the boiling water to a warm concentrated solution of corrosive sublimate in 35% alcohol for three hours (puncturing the body wall just before removing to this solution); then wash in 75% alcohol; then transfer to 75% alcohol to which a few drops of tincture of iodine has been added, to extract the corrosive sublimate, then wash in clear 75% alcohol and transfer to 85% alcohol for keeping. Do not use metal instruments in handling material fixed in corrosive sublimate. Put specimens in at least ten times their own bulk of the various solutions used. Keep in corked shell vials or large homeo vials.

For more detailed account of killing and fixing methods for insects, with reference to other fixing agents and special cases, see Comstock and Kellogg's *Elements of insect anatomy*, chap. VIII (p. 121-139), 1901; for exhaustive account of many killing and fixing agents (for miscellaneous animals) see Lee's *Microtomists' vade-mecum*. Also see these two references for more detailed consideration of the subjects of the following paragraphs.

Hardening, dehydrating, and clearing.—When ready to carry material further, select from the stock of properly killed and fixed specimens (preserved in 85% alcohol) the particular specimens desired to study and transfer to 95% alcohol for from 12 to 24 hours; then to absolute alcohol for from 12 to 24 hours; then to a half-and-half mixture of absolute alcohol and cedar-wood oil (or xylol). Pour the oil slowly into the vial containing the specimens in absolute alcohol; the oil and alcohol will remain distinct at first, the specimens keeping in the alcohol; as the two liquids gradually mix the specimens will become gradually (and hence safely) infiltrated with the new mixture. Leave in this mixture for from 12 to 24 hours. Transfer to pure cedar-wood oil (or xylol); leave from 12 to 24 hours. The specimens are now ready to be infiltrated with and imbedded in paraffine preparatory to cutting by the microtome.

Infiltrating and imbedding.—Remove specimens from pure cedar-wood oil, in which they may remain without injury indefinitely if for any reason the work must be interrupted, into cedar-wood oil into which about half the same bulk of paraffine shavings have been dropped and allowed to dissolve. This mixture should be kept in a watch glass or small dish at a temperature of about 45° C. To do this keep the dish in the paraffine oven or at the back end of an imbedding triangle. (Paraffine oven or imbedding triangle may be obtained of dealers in microscopic supplies.) Remove specimens from mixture of paraffine and cedar-wood oil after from three to six hours, depending on size and thickness of body wall of specimens, to melted pure paraffine of 54° C. melting point. This paraffine must be kept melted in paraffine oven or on imbedding triangle. The temperature should not be allowed to go up much higher than the melting point of the paraffine and never to fall below it

until the infiltrating is complete. This infiltration with pure paraffine will require from three to twelve hours, depending upon the size of specimens, and character of body wall. If it is necessary to interrupt the infiltration, the specimens in the melted paraffine should not be allowed to cool slowly but the paraffine should be hardened quickly by placing the paraffine dish on the surface of cold water and plunging it beneath the surface as soon as a firm film forms over the top of the paraffine. The paraffine can later be gradually melted and the infiltration proceeded with. When ready to imbed, pour some melted paraffine into a small paper boat or into a watch glass and transfer the specimens into this boat or watch glass, orienting them with a warm needle. Cool the paraffine quickly by putting boat or watch glass into cold water. (Do not plunge beneath surface of water until film forms on top of paraffine.) After cooling the paraffine, remove paper from around the block, or cut the block out from the watch glass, and wrap up in paper or put in a vial properly labeled. The specimens in these blocks may be kept indefinitely.

Cutting.—The work of cutting sections with a microtome must be learned by observation and experience. The many kinds of microtomes make any general description of the process impossible. For cutting insect tissues, where the whole body is sectioned or where any part of the body wall has to be cut, a heavy and rigid microtome is necessary. The light, swift, wheel microtomes are not the best for such work. I have found the large, heavy machine known as Minot's New Automatic Microtome, with large knife rigidly fastened at both ends, the best instrument, of several tried, for work with insects. The fixed knife and sliding object-carrier automatically raised make possible the ribboning of sections, while the horizontal position of the knife and the arrangement for the adjustment by hand of the block for each cut make it possible to pay that special attention to each section necessary in particular cases. With this microtome I have made complete series of such heavily chitinized specimens as the pupae of blepharocerid flies or the heads of various adult insects. With hard paraffine and a rigid powerful microtome strongly chitinized insect cuticle can be successfully cut without distorting or tearing the soft tissues lying next to it. For the study of the histolytic and histogenetic phenomena in the pupae of insects with complete metamorphosis it is necessary to make uninterrupted series of complete body sections including the heavy pupal cuticle. Hence the necessity of having in the entomological laboratory a microtome capable of such strenuous work.

The sections as cut may be transferred by brush or forceps or needle to a sheet of paper until the cutting is finished, or may be put directly on the slide. The slide should be well cleaned and dried and then smeared over with (almost) the thinnest possible coating of Mayer's albumen fixative. Arrange the sections in regular order in lines transverse or longitudinal to the slide, and when it is

covered (leaving always 1 sq. in. at one end for the label, to be put on later) gently flow enough distilled water from a pipette over it to float up all of the sections. Put the slide in a *safe* place to allow the water gradually to evaporate and the sections to dry thoroughly: they will, presumably, have spread out and dried perfectly flat and unfolded against the thinly smeared surface of the slide. Now gently heat the slide over a small flame until the paraffine of the sections has melted and thus further flattened and settled the sections against the glass surface. Let the paraffine harden, and put the slide into a small glass jar of pure xylol.

Clearing, staining, and mounting.—The xylol will dissolve away the paraffine in and enclosing the sections; leave slide in xylol for at least fifteen minutes; even a longer time is better. Then transfer to absolute alcohol for fifteen minutes to remove the xylol; then to 95% alcohol for from five to ten minutes; then to 75% alcohol for five minutes; then to 50% alcohol for five minutes; then into the alcoholic staining solution. There is a host of stains, some simple, some complex, some for general use, some for very particular and limited use. The beginner wants a simple stain for general use; and if he can get it in alcoholic solution, acidulated, he is relieved from carrying his slide through three or four more little jars containing, variously, water and acidulated alcohol. Transferring the slides through the alcohol series of lessening strength is simply to prevent the dangerously violent diffusion currents which are set up when an object saturated in strong alcohol is brought directly into weak alcohol. And if a stain in aqueous solution is used the series has obviously to be a longer one. For the beginner I recommend the use of Ehrlich's acid haematoxylin as a thoroughly satisfactory general stain. It is strong, staining quickly; it is an alcoholic solution, saving running the slides down to water; it is acidulated saving the differentiating bath in acid alcohol. It is cheap, and is a sharp, clean, pleasantly colored stain.

Leave slide in this stain from two to five minutes; only experience will determine the actual time for each slide. Wash slide in 50% alcohol; transfer to 75% alcohol for ten minutes; then to 85% alcohol for five minutes; then to 95% alcohol for five minutes; then to absolute alcohol for ten minutes; then to xylol for ten minutes. With thin balsam ready, remove the slide from xylol and put three or four drops of the balsam on it, and carefully but quickly, so as to prevent drying of any part of the slide by evaporation of the xylol, put the long cover-glass on in such way as to drive out all air-bubbles. Keep slide gently heated in paraffine oven or on top of imbedding triangle for half a day so as to harden the balsam; label and tuck away.

Keep the xylol, alcohol, and stain in little cylindrical staining jars or shell vials $3\frac{1}{2}$ in. high by $1\frac{1}{2}$ or 2 in. in diameter with class tops ground on, or good corks. Have a double series of alcohol jars, one set for the slide in its passage

(descent) from the paraffine-removing xylol down to the stain, and the other for the passage (ascent) from stain to the final xylol for clearing and preparing for the balsam mounting.

A host of changes can be rung on this simple, general procedure of clearing, staining, and mounting; but the beginner had better close his ears to these tunes and his eyes to the fascinating pages of Lee's *Vade-mecum* and similar guides wherein a dozen score of rainbow stains are described, and such refinements of manipulation set forth as would take a decade to learn and would make not a naturalist, but a microscopist. We need to know the technic of histology only in so far as it is necessary to know it, only in so far as we can use it, and only as a means to an end: the end being the study of insect tissue, not that of the behavior of triple stains.

THE HEMIPTERA DESCRIBED BY PHILIP REESE UHLER. III.

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REDUIVIDAE.

ACANTHODESMA, 47-271

perarmata, 47-271 Japan.

APIOMERUS

repletus, 16-329 Cal.

CONORHINUS

maximus, 44-286 L. Cal.

protractus, 44-284 Cal.: San Diego; L. Cal.: Santa Cruz?

rubidus, 44-285 L. Cal.: Cape San Lucas.

HARPACTOR

ornatus, 47-269 Japan.

ONCEROTRACHELUS

conformis, 43-211 Grenada.

ORTHOMETROPS, 52-508

decorata, 52-509 Md.: near Bladensburg; Pa.; N. J.: near Madison.

PINDUS

socius, 13-420 Id.: Snake river; Kans.; Dak.; Ariz.

PRIONIDUS, 21-23 = *Arilus* Hahn (1831).